Historical responses of distance between leaf teeth in the cool temperate rainforest tree Austral Mulberry *Hedycarya angustifolia* A. Cunn. from Victorian herbarium specimens

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Abstract

Distance between leaf teeth was examined in the cool temperate rainforest tree Austral Mulberry *Hedycarya angustifolia* A. Cunn. to determine whether this leaf character has the potential to be used as an environmental proxy. Photometric analysis was undertaken from Victorian herbarium-lodged specimens of *H. angustifolia* from 1852 to 2010. Regression analysis revealed no significant change in mean distance between leaf teeth in response to an increase of 0.9°C in mean annual temperature (MAT). From this preliminary analysis, distance between leaf teeth appears to be non-responsive to changes in MAT. It is possible that increases in MAT over the study period were insufficient to elicit a response in this leaf character, or that other environmental factors and/or intrinsic variation within this species may obscure any possible MAT signal. Future studies will be required to identify any probable drivers of this leaf character. (*The Victorian Naturalist* 131 (2) 2014, 36–39)

Keywords: Leaf teeth, *Hedycarya angustifolia*, mean annual temperature, Herbaria, environmental proxy

Introduction

Leaf morphology has long been found to respond to changing abiotic factors over environmental gradients in an attempt to maximise photosynthetic gains (CO_2 movement into the plant) and minimise transpirational losses (water loss from the plant via evaporation) (Bailey and Sinnott 1916; Givnish and Vermiej 1976). Changing abiotic factors such as increasing mean annual temperature (MAT) will increase transpirational losses and increase plant water stress, resulting in alteration to leaf form to reduce the effect of these stressors (Tricker *et al.* 2005). Altering the distance between leaf teeth may reduce transpiration rate by modifying the leaf boundary layer. The leaf boundary layer is a layer of static air over the leaf surface which provides a barrier to gas exchange (Schuepp 1993). Increasing the distance between leaf teeth does enhance boundary layer thickness and reduces transpirational losses by increasing the 'barrier width'.

If a leaf character responds in a predictable manner to an environmental variable, i.e. MAT, then this response could be employed as a proxy for that factor. Biological proxies have been successfully used to gain much information about previous climatic conditions, which may enhance our understanding of how future climate change may impact current ecosystems (Wing and Greenwood 1993; Royer *et al.* 2009). Recent studies have found leaf teeth characters, such as teeth number, size and area, are responsive to temperature and water availability (Hinojosa *et al.* 2011; Peppe *et al.* 2011; Royer *et al.* 2009). Scarr (1997) demonstrated that mean distance between leaf teeth in Austral Mulberry *Hedycarya angustifolia* A. Cunn. responded significantly to temperature factors but, to date, there have been no published studies examining the potential application of this leaf character as an environmental proxy.

Herbarium specimens provide a valuable resource that allows the examination of historical responses in foliar physiognomy (leaf form) over multi-generational time-scales. If there is a historical change in distance between leaf teeth in *H. angustifolia* across years of collection and if this correlates to increasing MAT, then this relationship may be assessed in multiple species as a standard environmental proxy measure in palaeoclimatological investigations. The aim of this study is to determine if distance between leaf teeth in herbarium-lodged Victorian specimens of the cool temperate rainforest tree, *H. angustifolia*, demonstrates historical change with particular focus on increasing MAT over the years of collection.

Methods

All leaf specimens were obtained from the National Herbarium of Victoria in Melbourne, Australia (MEL) spanning from 1852 to 2010. Sampling included creating a database for each herbarium sheet present in the MEL collections containing *H. angustifolia*. Information recorded from each herbarium sheet included (if available): sheet number, year of collection, latitude and longitude, elevation, branch tip present, flowers present and leaf number.

Due to limited research funding, the selection strategy was to sample one herbarium sheet per decade (if available) for the test species. Herbarium sheets were preferentially selected if a) sheets were from similar locations (to reduce genotypic variation), b) contained large numbers of leaves, and c) the specimen consisted of the branch tip with flowers present; which ensured that only sun leaves were sampled. Within each herbarium sheet, up to four leaves were photographed with a scale bar to be used for further photometric analysis (Fig. 1).

Images of sampled specimens were saved electronically as compressed TIFF files using the image conversion program Image Converter Plus. These TIFF images were loaded into ScionImage, an image analysis program, where the distance between every leaf tooth and its neighbour over the leaf margin were recorded in millimetres (mm). Individual distance measurements between teeth per leaf were recorded and the overall mean distance between leaf teeth per herbarium sheet was calculated. To determine if mean distance between leaf teeth significantly altered in response to year of collection and MAT, least squares linear regression was conducted using Predictive Analysis SoftWare v20 software (SPSS Inc.). All statistical analyses were conducted at an alpha level of 0.05.

Fig. 1. An example leaf from a herbarium specimen of *Hedycarya angustifolia* used for photometric analysis. Photo by J Cocking 2012.

Results

Herbarium sheets of *H. angustifolia* spanning 12 decades were sampled from 1852 to 2010 (Table 1), which corresponded to an increase in mean annual temperature (MAT) of 0.9°C over the last half of the previous century (CSIRO and BoM 2007). Mean distance between teeth varied from 6.55 mm in 1852 to 6.03 mm in 2010, with a maximum reading of 7.73 mm in 1929 and a minimum value 4.81 mm in 1947.

Least squares linear regression analysis demonstrated no significant relationship ($P > 0.05$) between mean distance between teeth and year of collection over the 158-year sampling period (Fig. 2a). In addition, no significant change ($P > 0.05$) was found in mean distance between teeth when the dataset was trimmed to examine the 0.9°C increase in MAT since the 1950s (CSIRO and BoM 2007) (Fig. 2b).

Discussion

Mean distance between leaf teeth was found non-responsive across years of collection or to increasing MAT in herbarium-lodged Victorian specimens of *H. angustifolia*. Therefore, based on this current dataset, the hypothesis

Table 1. Collection year of sampled herbarium sheets of *Hedycarya angustifolia* and associated mean distance between leaf teeth (mm) ± 1 standard error (S.E.).

Year	Mean	S.E.
1852	6.55	2.20
1888	7.00	2.00
1897	6.19	1.48
1901	5.87	1.74
1915	5.03	1.52
1929	7.73	2.42
1947	4.81	1.45
1952	6.06	1.56
1969	6.04	1.99
1979	6.76	1.62
2000	6.75	1.61
2010	6.03	1.11

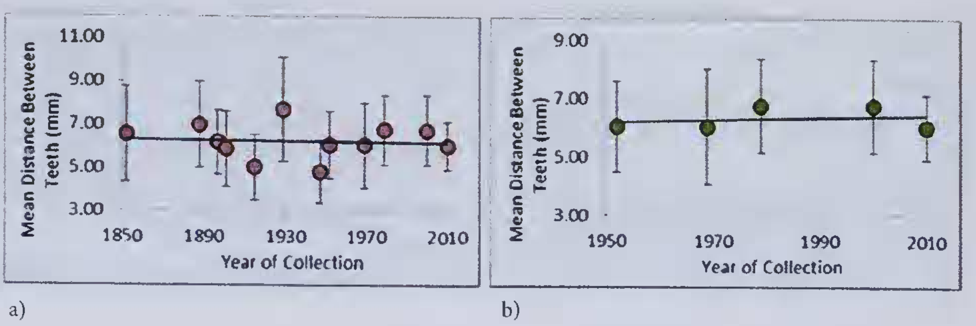

Fig. 2. Scattergrams with fitted linear trendlines for mean distance between leaf teeth versus a) year of collection from 1852 to 2010 ($y = -0.007x + 7.6027$, $R^2 = 0.0018$, $P > 0.05$) and b) year of collection from 1952 to 2010 ($y = 0.0044x + 2.4163$, $R^2 = 0.0698$, $P > 0.05$). Standard error bars ± 1 S.E.

that distance between leaf teeth would increase as a response to long-term increasing MAT can be rejected. It may then be inferred that there was no subsequent adjustment in leaf boundary layer width in response to this environmental stressor; however, when the dataset was trimmed to include only samples correlating to the 0.9°C increase in MAT since the 1950s the fitted trendline sloped upwards (Fig. 2b), indicating an increase in distance between leaf teeth, albeit insignificant. This lack of significant alteration to distance between leaf teeth may be attributed to, in part, the high degree of variation about the mean as can be seen from the standard error bar width (Fig. 2b).

Over the 158-year sampling period there is a large degree of scatter above and below the fitted trendline (Fig. 2a), which showed no significant change in distance between teeth over this temporal transect, a period that has experienced continual increase in climatic factors such as MAT and concentration of CO_2 (Etheridge *et al.* 1996; CSIRO and BoM 2007). This suggests that other environmental and/or intrinsic (within-species) factors may be the primary drivers controlling this leaf character. One collection variable that may impact upon leaf form is altitude at which the herbarium specimens were collected. Variation in altitude affects multiple environmental factors that influence leaf development, growth and photosynthetic potential (Crawford 1989; Scarr 1997). Environmental factors that alter with altitude are temperature, CO_2 contration, water and nutrient availability, soil type, wind speed and light levels (Körner and Cochrane 1985; Woodward 1986; Foreman and Walsh 1993). Aspect is another factor that will influence water availability at high altitudes, particularly on the leeward side of a range in the rain shadow, and thus leaf form may be affected.

Intrinsic variation may also dampen any environmental signature in leaf form within a dataset. Intrinsic variation is influenced by genotypic variability (i.e. alterations to gene expression) which results in differing phenotypes (physical features of the leaf). Thus, the influence of genotypic variation may be more significant in determining leaf phenotype than environmental variables, with considerable genetic variation being observed within a single location and within a single tree (Beerling and Chaloner 1993; Fordyce *et al.* 1995; James and Bell 1995). The lack of a significant alteration to the distance between leaf teeth in *H. angustifolia* to increasing MAT may be attributed to the large genotypic variation in this species.

Variation in environmental factors associated with altitude at which specimens grew, and intrinsic variation, may be mitigated by appropriate sampling strategies, that is, by sampling specimens collected from the same area at a similar elevation; however, an inherent problem in using herbaria is that the earlier lodged specimens lack comprehensive collection information on herbarium sheets. As a result, a researcher cannot completely remove all confounding variables associated with varying collection locations when using herbaria to establish a temporal transect.

Future research to establish if distance between leaf teeth in *H. angustifolia* can, in fact, respond to increasing MAT would be to employ growth chambers where plants can be reared in a controlled environment in which only temperature has been varied. Sampling of herbaria may be improved by trimming the dataset to include only herbarium sheets of known locations and altitude to reduce the potential impact of intrinsic variation upon this leaf character. Also, sampling herbaria over a latitudinal gradient (i.e. from Tasmania to Queensland) will establish a change in MAT of approximately 4°C, which may be sufficient to elicit significant alterations to leaf form. Finally, increasing the leaf number sampled per herbarium sheet may reduce scatter about the mean and, potentially, could result in a significant response being observed in distance between leaf teeth.

In conclusion, distance between leaf teeth from herbarium-lodged Victorian specimens of the cool temperate rainforest tree, *H. angustifolia*, does not appear to track changing MAT. But when the dataset was trimmed to include a period of rapid MAT increase, there was a positive trend with distance between leaf teeth, although not statistically significantly. Therefore, there may still be the potential to employ this leaf character as an environmental proxy but further research will be required to validate this.

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